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Antimicrobial Resistance among *Escherichia coli* Strains Isolated from Healthy and Septicemic Chickens

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Abstract: There is a clear association between heavy antimicrobial consumption in poultry industry and the recovery of resistant bacteria. This was a case-control study of 396 *E. coli* strains isolated from clinically affected broiler chickens and 132 strains from healthy controls to compare the antimicrobial resistance rates. Antimicrobial resistance testing of 525 avian *E. coli* strains isolated in Kashan-Iran showed very high levels of resistance to 11 antimicrobials tested, especially to trimethoprim-sulfamethoxazole (98.7%) and to ciprofloxacin (69.7%). The prevalence rate of resistant *E. coli* to ciprofloxacin and erythromycin in the samples isolated from chickens with colibacillosis was significantly higher than healthy controls. In addition, to prevent the emergence of cross-resistance with human enteric pathogens, controlled use of these antimicrobial agents in veterinary practice is recommended.

Key words: *Escherichia coli*, antimicrobial resistance, healthy chickens, septicemic chickens

INTRODUCTION

Colibacillosis in chickens is frequently manifested as a septicemia resulting in a subacute serositis characterized by fibrinoheterophilic infiltration producing a fibrinous pericarditis, perihepatitis, airsacculitis and pneumonia. It is one of the most serious threats to broiler chicken flock between 4 and 5 weeks of age with respiratory signs, omphalitis and septicemia in baby chicks causing high mortality rates. It is trasmitted through contamination of egg shell with pathogenic *E. coli* from poultry house or hatcher environment (AAAPG, 2005).

Antibiotics have been used on poultry husbandry farms to treat and control diseases in chickens and to improve chicken productivity (Peter, 1999). This practice is reported to have caused high resistance to antimicrobial agents in normal intestinal flora and pathogenic organisms of chickens (Tollefson *et al*., 1997; Manie *et al*., 1998). *E. coli* is commonly found in the intestinal tracts of animals, but only pathogenic serotypes cause avian collibacillosis (Aarestrup and Wegener, 1999; Gorbach, 2001). The transfer of resistant *E. coli* from chickens to humans is a common event, as has been demonstrated by several groups of researchers (Kanai *et al*., 1983; Van den Bogaard and Stobberingh, 1999). Antimicrobial agents are widely used in poultry husbandry in Iran as therapy for an infection or, in the absence of disease, in sub-therapeutic doses with the goals of growth promotion and enhanced feed efficiency. Multidrug-resistant strains of *E. coli* are prevalent in both human and animal isolates in different parts of the world and Iran, too (Nazer, 1980; Blanco Jesus *et al*., 1997; Al-Ghamdi Mastour *et al*., 1999). To look into these issues with more details, the current study was designed to evaluate the frequency of resistant *E. coli* isolated from septicemic broiler chickens and healthy controls.

MATERIALS AND METHODS

This was a case-control study of 396 *E. coli* strains isolated from clinically affected broiler chickens and 132 strains from healthy controls were collected in 20 commercial poultry husbandry fields in province of Isfahan (Kashan, Iran) in 2004. All strains isolated from diseased chickens came from confirmed cases of collibacillosis in which bacteria were obtained in culture from liver and heart tissues. Fecal strains were isolated from the cloacal contents of healthy chickens. Antimicrobial susceptibilities were assessed for amikacin, ampicillin, chloramphenicol, ciprofloxacin, gentamicin, tobramicin, doxycycline, nalidixic acid, erythromycin, nitrofurantoin and trimethoprim/sulfamethoxazole by the standard disk diffusion method in Muller-Hinton agar with disks provided by Difco and Biomerieux according to

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standards developed by the National Committee for Clinical Laboratory Standards (NCCLS, 1999), which its name recently changed to CLSI (Clinical Laboratory Standards Institute), guidelines. *E. coli* ATCC 25922 and *E. coli* ATCC 35218 were quality-control organisms. Three samples of healthy controls were excluded from the study. The statistical procedures were: Fischer's exact test and Chi-square. We considered differences significant at $p<0.05$. The ethical committee of the Kashan University of Medical Sciences approved the study.

RESULTS AND DISCUSSION

The resistance frequencies for 11 antimicrobial agents tested are shown in Table 1. As the Table 1 indicates, resistance frequency rates were very high. The resistance rates for 525 *E. coli* isolates were very high to amikacin and doxycycline (99.4%), nalidixic acid (99%), followed by ampicillin, trimethoprim-sulfamethoxazole (SXT), erythromycin and chloramphenicol. Ciprofloxacin showed high level of resistance rates (69.7%). The antibiotic resistance rates to tobramycin and gentamicin were at moderate level (11.6 to 12.6%). The resistant rates of *E. coli* to ciprofloxacin ($p<0.00002$) and erythromycin ($p<0.014$) in the samples from chickens with collibacillosis was significantly higher than healthy controls.

We have investigated the resistance of the large proportion of *E. coli* isolated with reduced susceptibility to antimicrobials could be the frequent exposure of chicken to abundant use and misuse of these drugs in commercial poultry production and colonized the resistant *E. coli* to 11 antimicrobial agents commonly used in the poultry industry and/or for human medicine in Kashan, Iran. The isolated *E. coli* from chickens clearly demonstrated high resistance rates to all tested antibiotics commonly used in the poultry industry.

In vitro antibiotic sensitivity results obtained in this study agreed with several various reports (Blanco Jesus *et al*., 1997; Al-Ghamdi Mastour *et al*., 1999), which have indicated increasing incidence rates of antibiotic resistant *E. coli* strains isolated from healthy controls and diseased chickens (Blanco Jesus *et al*., 1997). However, the high percentage of *E. coli* strains that were resistant to amikacin, doxycyline, nalidixic acid, trimethoprim/sulfamethoxazole and erythromycin in present study was surprising. An interesting finding in this study was that the percentage of *E. coli* resistant strains to erythromycin and ciprofloxacin (69.7%) in diseased group were significantly higher than in control. Lee *et al.* (2005), reported high resistance to ciprofloxacin (CIP; 60.2%), enrofloxacin (ENO; 73.4%) and norfloxacin (NOR; 60.2%) in one hundred and twenty-eight isolated *E. coli* from chicken.

The existence of high resistant *E. coli* in this study, like other results of other researches is alarming and aware us to use these drugs according to results of antibiogram tests and use the narrow spectrum instead of broad spectrum antibiotics (Blanco Jesus *et al*., 1997; Al-Ghamdi Mastour *et al*., 1999). Kijima-Tanaka *et al.* (2003), reported higher resistance rates in *Escherichia coli* isolated from food-producing animals in Japan against oxytetracycline and dihydrostreptomycin, followed by ampicillin and kanamycin. Resistance was more frequently showed among broiler isolates, followed by isolates from pigs. Almost 10% of broiler isolates were resistant to fluoroquinolones and extremely high MICs (100 mg L^{-1}) were observed. As Asai *et al.* (2005), mentioned the antimicrobial susceptibility of the isolates was examined against 7 classes of 11 antimicrobials. The rates of antimicrobial resistance among the isolates were found to correlate significantly with the usage of antimicrobial agents in cattle, pigs and broiler and layer chickens. Therefore, the overall usage of veterinary antimicrobials appears to contribute to the appearance of antimicrobial resistance in *E. coli* isolates from apparently healthy food-producing animals. In other study isolated *E. coli* from diseased piglets (n = 89) and chickens (n = 71) in China were characterized for antimicrobial susceptibility. The isolates displayed resistance to nalidixic acid (100%), tetracycline (98%),

Table 1: Antimicrobials resistance among *Escherichia coli* strains isolated from healthy and septicemic chickens in Kashan-Iran in 2004

Antibiotic amt. (μg in disk)	Healthy No. (%)	Septicemic No. (%)	Total No. (%)	p-value
Ampicillin (10)	127 (98.4)	392 (99.0)	519 (98.7)	0.638836
Gentamicin (10)	13 (10.1)	53 (13.4)	66 (12.6)	0.406050
Tobramycin (10)	11 (8.5)	49 (12.4)	60 (11.6)	0.301468
Amikacin (30)	128 (99.2)	394 (99.5)	522 (99.4)	0.571653
Doxycycline (30)	128 (99.2)	394 (99.5)	522 (99.4)	0.571653
Erythromycin (15)	121 (93.8)	393 (99.2)	510 (97.1)	0.014274
Nalidixic acid (30)	127 (98.4)	298 (75.3)	520 (99.0)	0.600754
Ciprofloxacin (5)	68 (52.7)	374 (94.4)	366 (69.7)	0.0000023
Chloramphenicol (30)	116 (89.9)	364 (91.9)	490 (93.3)	0.112967
Nitrofurantoin (300)	125 (96.9)	390 (98.5)	489 (93.1)	0.081295
SXT (25)	128 (99.2)	390 (98.5)	518 (98.7)	≈1

Total incidences of resistance of strains from healthy (n = 129) and septicemic (n = 396) chickens were 1092 and 3490, respectively (total for both groups, 4582). Incidences of resistance per strain were 8.46 and 8.8 for healthy and septicemic groups, respectively (average for both groups, 8.6)

sulfamethoxazole (84%), ampicillin (79%), streptomycin (77%) and trimethoprim-sulfamethoxazoole (76%). Multiple-antimicrobial-resistant *E. coli* isolates, including fluoroquinolone-resistant variants, are commonly present among diseased swine and chickens in China (Yang *et al*., 2004).

Ninety-five avian pathogenic *E. coli* (APEC) isolates recovered from diagnosed cases of avian colibacillosis from North Georgia. Multiple antimicrobial-resistant phenotypes (> or = 3 antimicrobials) were observed in 92% of *E. coli* isolates, with the majority of isolates displaying resistance to sulfamethoxazole (93%), tetracycline (87%), streptomycin (86%), gentamicin (69%) and nalidixic acid (59%) (Zaho *et al*., 2005).

In present study the large proportion of *E. coli* isolated with reduced susceptibility to antimicrobials could be result of the frequent exposure of chicken to abundant use and misuse of these drugs in commercial poultry production and colonized the resistant *E. coli*. The high recovery rate of antimicrobial resistant *E. coli* from broilers in Iran was troubling, but not surprising. Given the routine application of the antimicrobials at subtherapeutic doses for prophylactic and therapeutic purposes by farmers, without prescription and for treatment by veterinary prescription in absence of documented laboratory findings.

In this study, current frequent usage of particular antibiotics in chickens seemed to correlate not only with high prevalence of resistance to those antibiotics, but also with a higher prevalence rates of drug resistance in the population of chickens. Frequent usage of an antibiotic in the past was also associated with relatively high prevalence of resistance in this population.

In conclusion, the increasing problem of bacterial resistance due to rational usage of antibiotics including the implementation of a veterinary antibiotic policy is of utmost importance to safegurad the efficacy of veterinary antibiotic therapy for the future and to minimize the public health risks from veterinary practice. Surveillance is an essential part of antibiotic administration policy and should consist of the regular or continuous monitoring of antimicrobial resistance.

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